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Capillary Electrophoresis Chips with Integrated Electrochemical Detection

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Capillary electrophoresis systems with integrated electrochemical detection have been microfabricated on glass substrates. Photolithographic placement of the working electrode just outside the exit of the electrophoresis channel provides high-sensitivity electrochemical detection with minimal interference from the separation electric field. Microchip electrophoretic separations of neurotransmitters in under 100 s exemplify the good resolution and attomole detection sensitivity of these devices. Using indirect electrochemical detection, high-sensitivity DNA restriction fragment and PCR product sizing has also been performed. These microdevices match the detector's size to that of microfabricated separation and reaction devices, bringing to reality the lab-on-a-chip concept.

Microfabrication has the potential to revolutionize chemical and biological analysis, just as it has revolutionized electronics and computing. Recent applications of microfabrication in chemistry and biology include the production of micrometer-scale lipid bilayer arrays,[1] microlithographic arrays of obstacles for electrophoretic fractionation,[2] complex surface patterns,[3] and oligonucleotide hybridization arrays for DNA diagnostics and sequencing.[4] The recent development of microfabricated capillary electrophoresis (CE) chips and their use for rapid biological and chemical analysis[5−8] demonstrates that capillary electrophoresis[9−11] is also enhanced through miniaturization. Clearly, miniaturization and microfabrication increase the speed and information content of analyses, allow convenient integration of processes, and reduce sample volumes.

The ultimate development of a chemical analysis microprocessor on a single chip will depend on the integration of miniaturized chemical reaction, separation, and detection systems. Efforts toward integration have led to microdevices that can perform DNA restriction digestion and subsequent size-based separation,[12] carry out polymerase chain reaction (PCR) amplification followed by amplicon sizing,[13] and perform cell sorting and membrane lysis of selected cells.[14] However, less progress has been made toward integration of miniaturized detection. While laser-induced fluorescence provides sensitivity even to the single-molecule limit,[15−17] having a detection system that is orders of magnitude larger than a microfabricated analysis device reduces the benefits of miniaturization. Electrochemical methods[18] provide an alternative detection approach, offering both high sensitivity[19−21] and compatibility with microfabrication.[22−26] Miniaturized electrochemical detection has been used for cyclic voltammetry of less than nanoliter volumes [24,25] and to detect conventional CE separations.[22] Recently, an array of 100 microfabricated electrodes was used for spatially resolved electrochemical detection of neurotransmitter separations performed in a 0.6- or 8-μm-high, 1-cm-wide open-channel electrophoresis format.[23,26] We were therefore interested in the development of microfabricated CE chips with integrated injection and electrochemical detection as a means to further exploit the benefits of miniaturization and produce a completely integrated microanalysis device.

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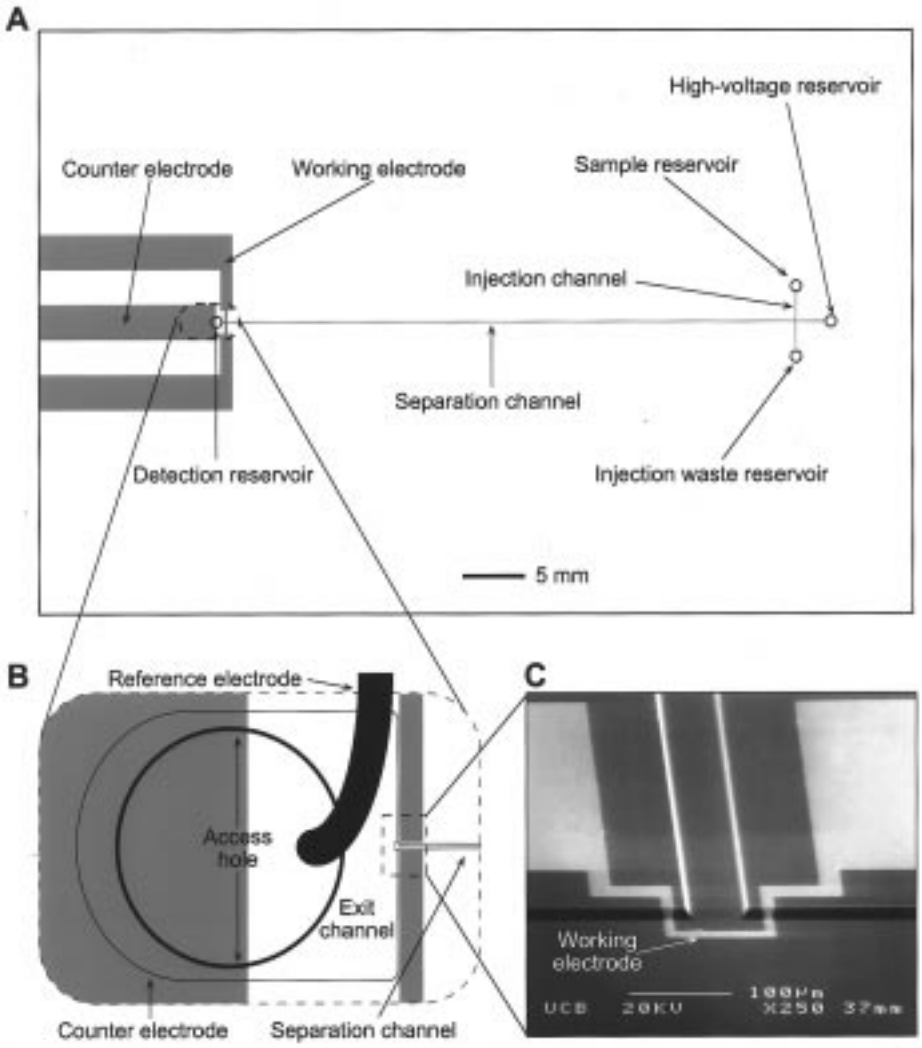

Figure 1. Capillary electrophoresis chip with integrated electrochemical detection. (A) Full chip view. Injection channel length, 5 mm; separation channel length, 50 mm; channel full width at half-depth, 46 μm; channel depth, 8 μm. The actual devices used had two independent electrophoretic analyzers on a 5 $\times$ 7.5 cm substrate. (B) Expanded view of the integrated electrochemical detector. (C) Scanning electron micrograph (140$\times$) of the detection region showing the location of the working electrode in the exit channel 30 μm beyond the end of the separation channel. Image has been rotated 90° for viewing clarity.

EXPERIMENTAL SECTION

Microfabrication. The design of our CE chips with integrated electrochemical detection is presented in Figure 1A. Capillaries were microfabricated using previously described photolithography, wet chemical etching, and thermal bonding methods.[8,27,28] The 70-μL detection reservoir was defined by a 2.5-mm-thick sheet of cured poly(dimethylsiloxane) (Sylgard 184, Dow Corning) having a 6-mm-diameter hole in the middle that was placed over the 0.8-mm-diameter drilled access hole. The other buffer reservoirs were formed by inserting cutoff 10-μL pipet tips into the drilled access holes; Pt wires placed in the reservoirs provided electrical connections to the channels. Photolithographically defined, rf plasma sputtered Pt working and counter electrodes (2600 Å with 200-Å Ti adhesion layer), and a Ag/AgCl reference electrode (placed in the access hole as close as possible to the working electrode), comprised the electrochemical detector. Chips either were fabricated with electrodes deposited on an etched glass plate and bonded to a drilled top substrate or with electrodes deposited on an unetched glass plate and bonded to an etched substrate having drilled holes. Figure 1B shows the photolithographically defined channel pattern used to minimize the effect of the separation electric field on electrochemical detection. The separation channel widened from ≈50 to 1000 μm just before the working electrode; the decreased resistance of the exit channel lowered the electric field in the detection region. Figure 1C presents a scanning electron micrograph of the detection region of an unbonded device; the 10-μm-wide working electrode had dual leads for ease of connection and extended 30 μm into the 1-mm-wide exit channel. Microfabrication technology is particularly advantageous for this chip design because it allows facile, precise, and stable placement of the working electrode in the exit channel just beyond the end of the separation channel. This decouples the detector from the electrophoresis voltage and eliminates the problem of electrolysis in the separation channel.

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Electrophoresis Procedures. Capillary zone electrophoresis separations were carried out in uncoated channels to enable electroosmotic flow; the electrophoresis buffer was 25 mM 2-(*N*-morpholino)ethanesulfonic acid, 1 mM Cl^-, pH 5.7. Neurotransmitter stock solutions (100 mM) were made by dissolving dopamine, epinephrine, or catechol (Sigma, St. Louis, MO) in 0.1 M $HClO_4$. Neurotransmitter samples were diluted to the desired concentration in electrophoresis buffer. Injection was performed by applying −120 V to the injection waste reservoir for 90 s while maintaining all other reservoirs at ground potential. Separation was performed with the separation voltage (V_s) applied to the high-voltage reservoir, 0.75 V_s applied to the sample and injection waste reservoirs, and the detection reservoir maintained at ground.

The DNA separation matrix contained 0.75% hydroxyethylcellulose, 40 mM Tris-acetate, 1 mM EDTA, 1 mM Cl^-, and 1 μM $Fe(phen)_3^{2+}$ (phen = 1,10-phenanthroline),[29] pH 8.3; channel surfaces were derivatized with linear polyacrylamide to suppress electroosmotic flow.[8] Injection was performed by applying +120 V to the injection waste reservoir for 20 s while maintaining the sample reservoir at ground and with the other two reservoirs floating. Separation was carried out by applying −800 V to the high-voltage reservoir while maintaining the detection reservoir at ground and with the other two reservoirs floating. ΦX174 *Hae*III restriction digests (New England Biolabs, Beverly, MA) diluted to a concentration of 1 ng/μL or 500 pg/μL and a *Salmonella* PCR product[13,30] diluted 1:200 were used as the samples for DNA separations and were prepared in 0.1 mM Tris-Cl^-, 0.01 mM EDTA, pH 8.2.

Electrochemical Detection. An amperometric detector (Bioanalytical Systems, LC-4C, West Lafayette, IN) was used for electrochemical detection. A 250-μm-diameter Ag wire was oxidized in 1 M Cl^- and then placed in the access hole, which contained buffer with 1 mM Cl^-, to form the Ag/AgCl reference electrode. The working electrode was maintained at +800 mV vs the Ag/AgCl reference electrode for both neurotransmitter and DNA separations. To maintain signal reproducibility for the neurotransmitter separations, the working electrode was cleaned before each run by applying 0 mV vs the reference electrode for 70 s prior to injection. Cleaning the working electrode was not necessary for DNA separations. Output signal from the detector was filtered (Stanford Research Systems, SR560, Sunnyvale, CA) to remove high-frequency noise and sampled at 5 Hz by a Macintosh Centris 650 computer with a NB-MIO-16-XL analog-to-digital converter and LabView software (National Instruments, Austin, TX).

RESULTS AND DISCUSSION

The minimum spacing between the working and reference electrodes was determined by the location of the access hole, and the distance between these two electrodes had a substantial effect on the performance of the integrated electrochemical detector. In Figure 2A this spacing was 600 μm, and V_s produced more interference than in Figure 2B where the spacing was only 300 μm. Separations of dopamine at 300 V (60 V/cm) in a chip with 600-μm spacing exhibited a flat baseline. Increasing V_s in 50-V increments to 450 V (90 V/cm) resulted in decreased signal and a sloping baseline. For chips with a 300-μm distance between the working and reference electrodes, the effect of V_s was much less pronounced, permitting faster separations at higher V_s. Dopamine separations at 600 V (120 V/cm) had a flat baseline, and increasing V_s in 200 V increments resulted in only minor changes in the signal and baseline slope until V_s reached 1200 V (240 V/cm). This effect of V_s on the detection background is probably due to the difference between the potential in the buffer above the working electrode (determined by V_s) and the potential set at the working electrode by the potentiostat. On the basis of these results, we used chips with a 300-μm spacing between the working and reference electrodes and V_s = 800 V for subsequent experiments.

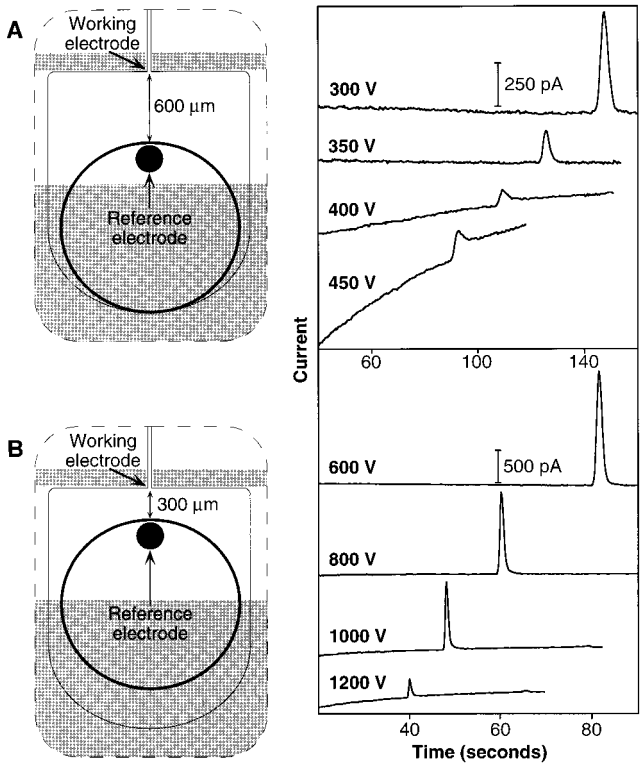

Figure 2. Effect of the distance from the reference to the working electrode on separations. (A) Reference electrode 600 μm from the working electrode. Electrophoretic separations of 1 mM dopamine at 300, 350, 400, and 450 V. (B) Reference electrode 300 μm from the working electrode. Electrophoretic separations of 1 mM dopamine at 600, 800, 1000, and 1200 V.

To characterize these devices, we first performed capillary zone electrophoresis separations of neurotransmitters. Traces A−C in Figure 3 present separations of dopamine, epinephrine, and catechol, respectively, in a microfabricated CE chip with integrated electrochemical detection. Figure 3D demonstrates the separation of a mixture of all three species in this device; dopamine and epinephrine differ in migration time by 2.8 s but are still baseline-resolved. The numbers of theoretical plates in this separation were 21 000, 17 000, and 12 000 for dopamine, epinephrine, and catechol, respectively. The signal from each of the three components as a function of concentration was linear in the range from 10 to 1000 μM, as shown in Figure 3E. Using the standard deviation of the signal (1.5 pA) from 55 to 70 s in a blank

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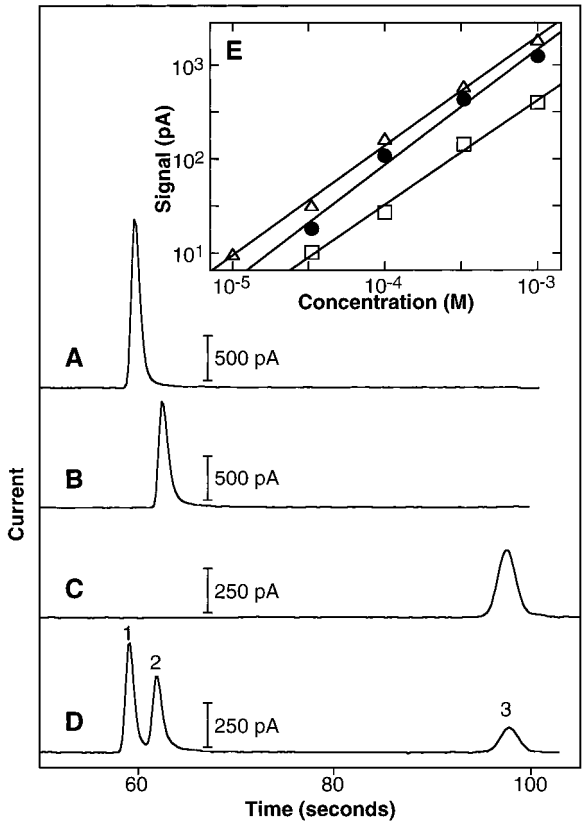

Figure 3. Electrophoretic analysis of neurotransmitters on CE chips with integrated electrochemical detection: (A) separation of 1 mM dopamine; (B) separation of 1 mM epinephrine; (C) separation of 1 mM catechol; (D) separation of 330 μM dopamine (1), epinephrine (2), and catechol (3). Injection, separation, and detection were performed as detailed in the Experimental Section. (E) Peak height in separations as a function of injected concentration of dopamine (Δ), epinephrine (●) and catechol (□). Points on the plot represent the mean signal obtained in three duplicate separations. The on-column limit of detection for dopamine was 66 amol.

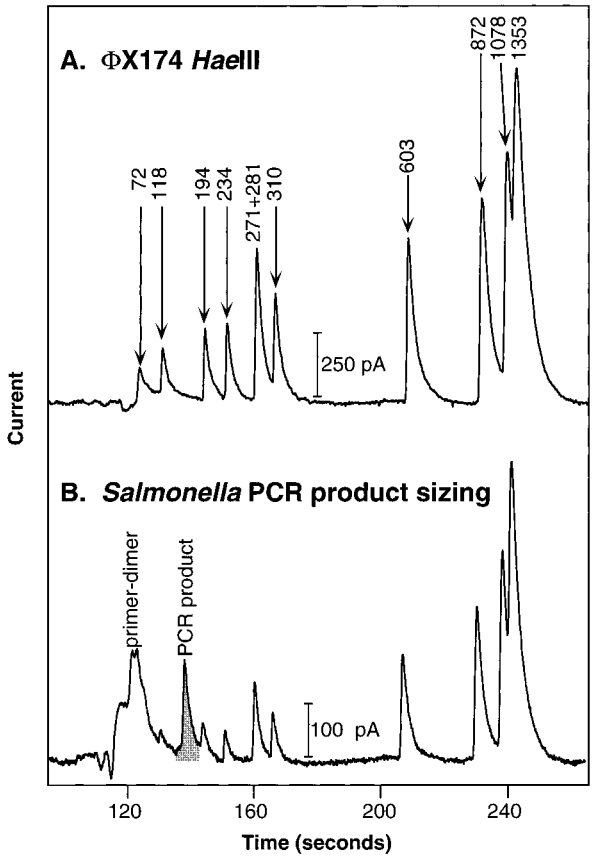

Figure 4. DNA analysis using a CE chip with integrated electrochemical detection. (A) Separation of a ΦX174 *Hae*III restriction digest (1 ng/μL); (B) separation of a 1:200 dilution of a *Salmonella* PCR product (shaded) and 500 pg/μL ΦX174 *Hae*III restriction digest. The current axis has been inverted in (A) and (B) to display the electropherograms with the peaks pointing up.

run, we determined limits of detection (signal-to-noise ratio, 2) of 3.7 μM for dopamine, 6.5 μM for epinephrine, and 12 μM for catechol. Since injection and separation were carried out with the potentials of all reservoirs controlled to counteract diffusional broadening,[7] the injection volume is defined by the volume of the intersection of the two channels (18 pL). Based on this estimate, the limit of detection of dopamine on-column was 66 amol, well within the expected range for electrochemical detection in CE.[18]

The general applicability of these CE chips would be enhanced by developing electrochemically active labels and detecting other biomolecules. As a first step in this direction, we have developed methods for separation and detection of DNA in microfabricated CE chips using an indirect electrochemical detection approach[31,32] with the electrochemically active intercalation reagent Fe(phen)$_3^{2+}$.[29] The constant background current from free Fe(phen)$_3^{2+}$ in the separation buffer decreases when DNA−Fe(phen)$_3^{2+}$ complexes migrate through the detection region, so the passage of DNA is indicated by transient dips in the background current. Figure 4A shows the separation of a 1 ng/μL ΦX174 *Hae*III restriction digest in a microfabricated CE chip with integrated electrochemical detection. Figure 4B further demonstrates that these microdevices have the sensitivity and resolution to size PCR products; in this example, a 159-bp amplicon from *Salmonella*[13,30] was sized to 161 bp vs the ΦX174 *Hae*III peaks. The minor peak tailing observed in these separations is characteristic of indirect detection when the electrophoretic mobilities of the analyte and the indirect detection species are not the same[31] and is not a limitation of the injection, separation, and detection methodology shown here.

Injection of 50 ng/μL ΦX174 *Hae*III dissolved in electrophoresis buffer (under nonstacking conditions) yielded a signal of 190 pA for the 603-bp band. Based on an injection volume of 125 pL calculated from our previously estimated injection plug length of 330 μm,[8] 700 fg (1.8 amol) of the 603-bp fragment was injected; therefore, the estimated detection limit (signal-to-noise ratio, 2) was 28 zmol (17 000 molecules) for this fragment. These results indicate that CE chips with integrated electrochemical detection may be used for high-sensitivity biochemical assays that are competitive with traditional fluorescence detection methods.[7,12,33]

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PROSPECTS

The concept of fabricating an entire chemical analysis process on a chip has rapidly progressed over the past few years.[5,7,8,12−14] However, with the large optical detection systems that are typically utilized at present, these devices may not be able to reach their full potential as portable analysis tools. With the work presented here we have bridged the macro- to microgap by microfabricating both the separation and detection systems on a single chip. Development of electrochemical labels for detection of chemical and biological analytes will pave the way to a host of applications including health care diagnostics and pathogen detection. The use of direct labeling methods and multipotential electrochemical detectors should also enable multiplex electrochemical detection analogous to multiwavelength fluorescence detection. Now that complete devices can be microfabricated, by improving the layout and packaging it may even be feasible to make chemical analysis microprocessors where the detection and computer circuitry are also integrated on-chip. With these improvements, integrated microanalysis devices should be capable of probing for biological signatures in a variety of challenging locations, including even extraterrestrial environments.[34]

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